2. Micrococcin is almost insoluble in water. In ethanol-water mixtures its solubility rises to > 150 mg./ml. at 70–80 % ethanol, then falls to less than 10 mg./ml. in absolute ethanol.

3. It decomposes at 222–228°. $[\alpha]_D^{21°} = +116°$. It has a broad absorption in the ultraviolet and many bands in the infrared. An ethanolic solution has a strong purple fluorescence in near-visible ultraviolet light.

4. Both fluorescence and ultraviolet absorption are reduced by traces of copper and partly restored by BAL.

5. The molecular weight appears to be somewhat over 2000, and elementary analysis gave C, 49–49·5; H, 4·6; N, 13·9; S, 15·9 %. It gives a positive ninhydrin reaction only after acid hydrolysis, but this seems to be due to ammonia, not amino-acids. Nothing is known of its chemical nature.

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REFERENCES

Craig, L. C., Golumbic, C., Mighton, H. & Titus, E. (1945). *J. biol. Chem.* **161**, 321.

Feigl, F. (1946). *Qualitative Analysis by Spot Tests*, 3rd English ed. New York: Elsevier.

Heatley, N. G., Gowans, J. L. & Florey, H. W. (1951). *Brit. J. exp. Path.* (in the Press).

Heatley, N. G., Kelly, B. K. & Smith, N. (1951). *J. gen. Microbiol.* (in the Press).

Kelly, B. K., Miller, G. A. & Hale, C. W. (1951). *J. gen. Microbiol.* (in the Press).

Markham, N. P., Heatley, N. G., Sanders, A. G. & Florey, H. W. (1951). *Brit. J. exp. Path.* **32**, 136.

Markham, N. P., Wells, A. Q., Heatley, N. G. & Florey, H. W. (1951). *Brit. J. exp. Path.* (in the Press).

Sanders, A. G., Florey, H. W. & Wells, A. Q. (1951). *Brit. J. exp. Path.* (in the Press).

Su, T. L. (1948). *Brit. J. exp. Path.* **29**, 473.

Metabolic Effects of Electrical Stimulation of Mammalian Tissues *in vitro*

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It has been found that electrical stimulation of slices of guinea pig brain cortex *in vitro* gives rise to large increases in the rate of oxygen consumption and aerobic glycolysis (McIlwain, 1951; McIlwain, Anguiano & Cheshire, 1951).

In order to test the scope of the new method, as to whether the response to electrical stimulation is confined to nerve tissue or is a property of other tissues, the technique has now been applied to rat diaphragm, and guinea pig lung and kidney slices.

Isolated whole frog muscle responds to electrical impulses by increased oxygen consumption which is several times that of the resting muscle (Meyerhof & Schulz, 1927; Fenn, 1927; Gemmill, 1934). Also Millikan (1937) has shown that *in vivo* cat muscle, during active contraction, can respire at a rate nearly twenty times that of the muscle at rest. Little work has been done to see what response is made to electrical impulses *in vitro* by other mammalian tissues.

METHODS

Guinea pig kidney cortex and lung slices, 0·35 mm. thick, were cut by means of a Stadie-Rigg's cutter. The thickness of slices was less than the maximum limit stated by Warburg (1930) and Field (1948) as being allowable for full oxygenation. Guinea pig-brain cortex slices were cut as described by McIlwain & Grinyer (1950). The diaphragms of young rats of about 100 g. were dissected out and the peripheral muscle portions used. According to Cohen (1949*a*) the diaphragms from animals of this size have a thickness of about 0·3 mm. The procedure in weighing tissues was that described by McIlwain (1951).

Krebs-Ringer phosphate solution, as described by Cohen (1949*b*), was used with the concentration of calcium reduced by half to prevent the formation of a precipitate. Glucose was added to give a concentration of 0·1 % (0·0056 M). Media were oxygenated at 37°.

The manometer flasks, of conical shape, were of about 18 ml. capacity; to some of them lead-in wires were inserted (type *A*, McIlwain, 1951). The tissues were fixed in small plastic and silver gauze tissue-holding electrodes (type *B*,

McIlwain, 1951). In most of the experiments, the control tissues were fixed in similar electrodes. The centre well contained 0·1 ml. 5N-KOH and paper to absorb CO_2. An experiment was made in stages as follows: (1) a preliminary equilibration period of 10–15 min.; (2) measurement of resting O_2 uptake over a period of 20–30 min. (period A); (3) measurement of O_2 uptake while the tissues were exposed to electrical impulses (period B); this treatment was omitted in the control experiments. The applied voltage was 2·5 V. (as measured by a moving coil meter) supplied by a variable transformer run from the 50-cycle a.c. mains. The current was about 10–25 ma. The greater proportion of this current was short circuited through the saline and did not pass through the tissue: this was tested in one instance by the currents with and without the saline present. The currents were respectively 13 and 0·1 ma. In a few experiments alternating pulses from a 2 or 4 μF. condenser, charged from a 12 or 24 V. battery, as described by McIlwain (1951), were used as an alternative form of impulse. The time constant of discharge was between 1 and 2 msec.

Lactate was measured by the method of Barker & Summerson (1941).

RESULTS

Normal respiration. The means of all initial (period *A*) rates of oxygen uptake are given in Table 1. These include the values for the controls which were treated in exactly the same way as the test material in this initial period.

All tissue weights quoted are the initial wet weights. These have been used in preference to the final dry weights, for there is evidence (Bach, 1944; Cutting & McCance, 1946) that leaching of the solid matter of tissue slices occurs in physiological salines. In order to compare the rates of oxygen consumption with those of other workers who expressed their results upon post-experimental dry weight of tissue, the dry/wet weight ratios for fresh slices have been determined. The values are: brain cortex, 0·16; lung, 0·15; kidney cortex, 0·20; diaphragm, 0·26. The corresponding rates of oxygen uptake are shown in Table 1. It is seen that they are

Table 1. *Rate of oxygen consumption during initial period*

(Incubation at 37° in O_2 of slices suspended in modified Krebs-Ringer phosphate solution.)

Animal	Tissue	No. of animals	No. of determinations	Initial oxygen consumption (μmol./g. wet wt./hr.)	Initial oxygen consumption (μl./mg. dry wt./hr.)	Values recorded by other authors (μl./mg. dry wt./hr.)	Reference
Guinea pig	Brain cortex	4	12	73·1±4·9*	10·2	12·9	Wollenberger (1947)
						11·7	Edson & Leloir (1936)
						11·0	Jowett & Quastel (1937)
Guinea pig	Lung	11	52	40·5±1·6	6·0	7·0	Wollenberger (1947)
						6·1	Simon, Potts & Gerard (1947)
						6·1	Barron, Miller & Bartlett (1947)
Guinea pig	Kidney cortex	6	36	123·9±5·2	13·9	16·8	Wollenberger (1947)
						19·0 (μmol./g. wet wt./hr.)	Edson & Leloir (1936)
Rat	Diaphragm	12	23	68·8±2·9	5·9	75·9	Walaas & Walaas (1950)
						46·5	Gilmore & Samuels (1949)

* Standard error of the mean.

Table 2. *Oxygen consumption on exposure to* 2·5 V. *a.c.*

	Tissue slices	No. of determinations	Mean O_2 consumption (μmol./g. wet wt./hr.) Initial period	Mean O_2 consumption (μmol./g. wet wt./hr.) On exposure to current	Mean change (%)	Statistical significance of change caused by electrical stimulation
Brain cortex:	*a*, control tissue	6	72·0	64·4	− 8·0±5·2*	S ($P<0·01$)
	b, exposed tissue		74·2	112·0	+51·0±9·9	
Lung:	*a*, control tissue	26	40·5	41·0	+ 2·8±3·1	S ($P<0·01$)
	b, exposed tissue		40·5	47·1	+18·5±4·6	
Kidney cortex:	*a*, control tissue	18	120·0	112·5	− 6·0±1·4	NS
	b, exposed tissue		127·8	120·8	− 5·8±1·4	
Diaphragm:	*a*, control tissue	12	64·4	60·7	− 5·2±2·4	S ($P<0·01$)
	b, exposed tissue		73·4	101·8	+38·6±6·6	

* Standard error of the mean. S, Significant. NS, Not significant.

Table 3. *Alternating current on respiration of lung slices*

(Slices in modified Krebs-Ringer phosphate solution at 37°, exposed to 2·5 V. Figures in corresponding positions in the table refer to the same tissue slice. The variability as between different slices is probably due to the difficulty of obtaining a reliable wet weight of the spongy lung slice.)

Respiration during initial period (μmol./g. wet wt./hr.)				Respiration on exposure to impulses (μmol./g. wet wt./hr.)			
65	24	32	20	85	42	42	23
42	37	45	45	32	38	53	48
32	32	50	38	39	30	47	49
40	40	33	21	57	47	47	33
44	37	46	45	60	54	57	50
41	48	61	43	37	44	60	51
39	51			49	49		
Mean 40·5				Mean 47·1			

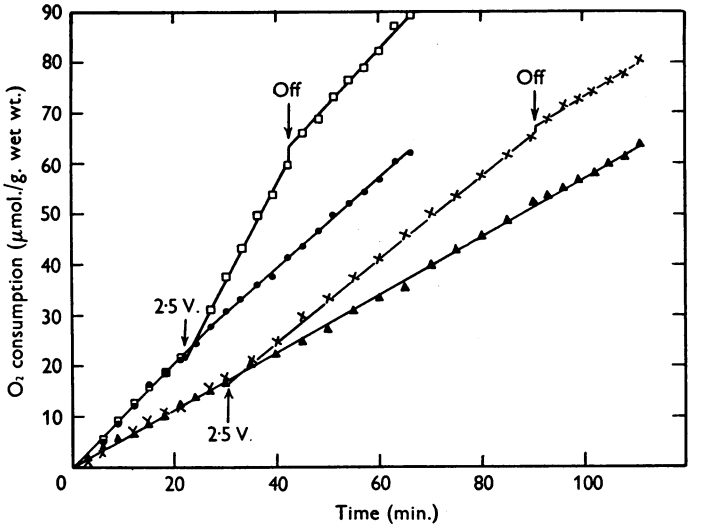

Fig. 1. The effect of 2·5 V. alternating current on the O_2 consumption of rat diaphragm and guinea pig-lung slices in modified Krebs-Ringer phosphate media. The instantaneous displacement on applying and switching off electric current is due to its heating effect (see McIlwain, 1951). Diaphragm: control, ●; exposed, □. Lung: control, ▲; exposed, ×.

rather lower than the values recorded by others. This may perhaps be accounted for by the reduction in weight due to leaching. There was found to be only a small difference between the oxygen consumption of a tissue held in the electrode and that of the same tissue in free suspension. Four experiments with diaphragm showed no appreciable difference under the two sets of conditions. In six experiments with brain cortex slices, the oxygen consumption of the tissue held in the electrode was 3 % greater, and with lung slices 12 % greater than that of the freely suspended tissue.

Influence of electrical impulses on respiration. The respiration rates of the tissues during the passage of electrical impulses are shown in Table 2. The statistical significance of observed increases over the normal values has been estimated by the '*t*' test.

The respiration of brain cortex slices showed a significant increase ($P < 0{\cdot}01$) over the control value during exposure to impulses. The same was true for diaphragm. The experiments with lung slices, when examined as a whole, also showed a significant increase ($P < 0{\cdot}01$). However, in contrast to brain cortex and diaphragm, which responded to the electrical impulses in every case, seven of the twenty-six lung slices showed no increase of respiration during the passing of the current, while with the others there was a graduation of response to a maximum of 75 %.

The individual results are given in Table 3. This variability of the response of lung is thought to be due to the presence of varying amount of muscle in the slices, which were cut from the apex at different depths. Kidney cortex, a tissue which contains no

muscle fibres, gave no response to electrical impulses; the respiration of both the experimental and the control slices fell by 6 % of their initial rate during period B.

McIlwain (1951) has reported that stimulation caused no permanent increase in the rate of oxygen consumption of brain cortex slices. In the present experiments the rate was similarly found to return nearly to its original value in those cases where a post-stimulation period was run. Examples for diaphragm and lung are shown in Fig. 1.

The alternative form of stimulation, by condenser discharge, was used in some experiments. In two cases lung slices showed no response, but in a third a 36 % increase in respiration was recorded. The respiration of diaphragm increased by 33 % as a result of condenser impulses. Kidney slices showed no response. These few experiments confirm the results obtained with alternating current.

Glycolysis. McIlwain (1951) has found that stimulation of brain tissue markedly increases its aerobic glycolysis. Therefore, it was decided to make similar tests with the different tissues used for the experiments on respiration. In order to facilitate the detection of any increase in glycolysis, the period of stimulation was arranged to be greater than half the total time that the tissues were in the manometric vessels. The results are given in Table 4.

Table 4. *Increase in lactic acid production by tissues on exposure to electrical impulses*

(All tissues suspended in modified Krebs-Ringer phosphate solution, pH 7·4, under O_2 at 37°, exposed to 2·5 V. a.c.)

Tissue	No. of pairs of determinations	Total experimental time (min.)	Period of exposure (min.)	Mean production of lactic acid by control slices (μmol./g. wet wt./ experimental time)	Mean production of lactic acid by exposed slices (μmol./g. wet wt./experimental time)	Mean change (%)	Significance of increase
Brain cortex	4	90–95	50–60	45·0±6·3	70·2±4·0	+56	S ($P<0·05$)
Lung	13	95–100	55–60	40·5±4·0	38·2±7·3	−6	NS
Kidney cortex	7	90–95	50–60	41·6±7·0	46·6±4·2	+13	NS
Diaphragm	9	80–90	50	22·6±4·3	33·1±4·8	+47	S ($P<0·05$)

S, Significant. NS, Not significant.

Lactic acid production by the control portions of diaphragm was greater than that reported by Gilmore & Samuels (1949), and Walaas & Walaas (1950), but in tissue slice experiments, lactic acid production can vary greatly with tissue/fluid ratios and with the duration of the experimental period. The stimulating current produced further amounts of lactic acid; the percentage increase was 47 %. The production of lactic acid by the control slices of kidney cortex was higher than had been expected on the basis of earlier observations of Dickens & Šimer (1929) who found that kidney cortex produced only traces of lactic acid under aerobic conditions. They stated that aerobic glycolysis can be caused in tissues due to slight mechanical or other damage without affecting the rate of respiration. In the present experiments the tissues were handled much more than those used by Dickens & Šimer, and this may account for the present high rate of glycolysis. A comparison between slices of kidney cortex suspended freely in the medium and similar slices held in electrodes, showed no difference in the rates of lactic acid formation. Thus it seems that the electrodes do not increase production of lactic acid by damage or by preventing adequate gas exchange. Kidney cortex slices showed no significant change in lactic acid production as the result of electrical impulses. Lung slices also were found not to change their aerobic glycolysis on stimulation. The rate of formation of lactic acid by brain cortex slices in control experiments as well as the increases produced by stimulation are within the range reported by McIlwain *et al.* (1951).

DISCUSSION

From the above experiments it can be seen that there are definite metabolic responses to electrical stimulation *in vitro* by both brain and diaphragm; a smaller response by lung and no response by kidney cortex. The lack of response by kidney suggests that the positive responses of other tissues are not due to some general reaction of the electric current. Curtis & Cole (1947) state that most organs of the body are not harmed by even a prolonged application of an alternating electric current and some organs, for example liver, show no measurable reaction to such a current. The failure of kidney to respond in the present experiments may have been due to two causes; either the tissue was totally unresponsive, or the impulses used were not suitable. However, impulses with various characteristics have been used without success, and it appears likely that the tissue was unresponsive, at least by the criteria so far used to measure response.

It would seem that, apart from brain tissue, a metabolic response to electric impulses occurs only

if muscle fibres are present. Diaphragm is a muscular tissue and lung contains muscle fibres while kidney contains none. This is further borne out by some preliminary experiments using heart slices, where a response was found, and liver or testis which showed none.

The oxygen consumption of rat diaphragm has also been increased by the action of chemical stimulants. Gordon & Heming (1944) found that injection of thyroxine into rats raised the oxygen consumption of the excised diaphragm by 29% from their normal respiration level of 5·6 μl. oxygen/mg. dry weight tissue/hr. A similar response was obtained by Gemmill (1947) on incubating rat diaphragm with theobromine derivatives. Caffeine, for example, raised the respiration by 29% from a basal rate of 53 μmol./g. wet weight tissue/hr. In assessing the results of the present experiments with rat diaphragm these results are of value in showing that under somewhat similar conditions to those used here, rat diaphragm is able to respond to several forms of stimulation.

SUMMARY

1. Tissue slices of guinea pig-brain cortex, kidney cortex and lung, and portions of rat diaphragm have been exposed to fluctuating electric currents *in vitro*.

2. These caused a mean increase of 51% in the rate of brain cortex respiration and an accompanying increase of 56% in aerobic glycolysis.

3. The mean increase in the respiration of diaphragm was 39% with an increase of 47% in aerobic glycolysis.

4. The impulses increased respiration of lung slices by 19%, but gave no increase in aerobic glycolysis.

5. No response to the impulses was obtained with kidney slices.

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REFERENCES

Bach, S. J. (1944). *Biochem. J.* **38**, 156.

Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.

Barron, E. S. G., Miller, Z. B. & Bartlett, G. R. (1947). *J. biol. Chem.* **171**, 791.

Cohen, P. P. (1949*a*). In *Manometric Techniques and Tissue Metabolism*, by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. 2nd ed. p. 115. Minneapolis: Burgess Publishing Co.

Cohen, P. P. (1949*b*). In *Manometric Techniques and Tissue Metabolism*, by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. 2nd ed. p. 118. Minneapolis: Burgess Publishing Co.

Curtis, H. J. & Cole, K. S. (1947). In *Medical Physics*, p. 798. Ed. Glasser, O. Chicago: Year Book Publishers Inc.

Cutting, M. & McCance, R. A. (1946). *J. Physiol.* **104**, 288.

Dickens, F. & Šimer, F. (1929). *Biochem. J.* **23**, 936.

Edson, N. L. & Leloir, L. F. (1936). *Biochem. J.* **30**, 2319.

Fenn, W. O. (1927). *Amer. J. Physiol.* **83**, 309.

Field, J. (1948). In *Methods in Medical Research*, p. 289. Ed. Potter, V. R. Chicago: Year Book Publishers Inc.

Gemmill, C. L. (1934). *J. cell. comp. Physiol.* **5**, 277.

Gemmill, C. L. (1947). *J. Pharmacol.* **91**, 288.

Gilmore, R. C. & Samuels, L. T. (1949). *J. biol. Chem.* **181**, 813.

Gordon, E. S. & Heming, A. E. (1944). *Endocrinology*, **34**, 353.

Jowett, M. & Quastel, J. H. (1937). *Biochem. J.* **31**, 565.

McIlwain, H. (1951). *Biochem. J.* (in the Press).

McIlwain, H., Anguiano, G. & Cheshire, J. D. (1951). *Biochem. J.* (in the Press).

McIlwain, H. & Grinyer, I. (1950). *Biochem. J.* **46**, 620.

Millikan, G. A. (1937). *Proc. roy. Soc.* B, **123**, 218.

Myerhof, O. & Schulz, W. (1927). *Pflügers Arch. ges. Physiol.* **217**, 547.

Simon, F. P., Potts, A. M. & Gerard, R. W. (1947). *J. biol. Chem.* **167**, 303.

Walaas, O. & Walaas, E. (1950). *J. biol. Chem.* **187**, 769.

Warburg, O. (1930). *Metabolism of Tumours*, p. 77. Ed. Warburg, O., translated by Dickens, F. London: Constable and Co.

Wollenberger, A. (1947). *J. Pharmacol.* **91**, 39.